

Applicants: Cristoph Hock et al.
Serial No.: 10/554,314
Filed: April 19, 2006
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Amendment to the Specification:

Please amend the subject specification as follows:

Please replace the title beginning on page 1, line 1 with the following amended title:

~~Method of Monitoring Immunotherapy~~ Tissue Amyloid Plaque
Immunoreactivity Assay

Please replace in the specification beginning on page 13, line 1, the following amended paragraph:

Tissue amyloid plaque immunoreactivity (TAPIR) assay: For the assessment of the ability of the human immune sera to react with *bona fide* β -amyloid plaques in brain tissue, a specific TAPIR assay, as disclosed in the present invention, was developed. Double transgenic mice (18 months old) expressing human APP and PS1 genes with pathogenic AD-causing mutations (APP^{SW}xPS1^{M146L}) were perfused and brains were fixed. Paraffin-embedded brains were sections (5 μ m) and incubated with human serum or CSF samples taken prior to the prime injection and 56.0 \pm 5.8 days (mean \pm S.D.) after the booster injection. Samples were used either undiluted or diluted 1:50 to 1:10,000 in 2% BSA and 5% donkey serum in PBS. After washing, human IgG bound to β -amyloid plaques were detected with cy3-conjugated donkey antibodies directed against heavy and light chains of human IgG (Jackson Labs, Bar Harbor, Maine). Fluorescent β -amyloid plaques on the sections were imaged through a 40x objective and a TRITC filter attached to a Nikon Eclipse E800 fluorescence microscope equipped with a Kappa PS 30C CCD camera. Images of all dilutions were acquired with standardized camera settings chosen to be well below the saturation of 255 arbitrary units (A.U.) in 8 bit mode. The Image J software (www.ncbi.nlm.nih.gov) was used to quantify the mean pixel intensities (range: 23 to 195 A.U.) of n-15 β -

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amyloid plaques per serum dilution. Averages of the means were used for both the standard curve and the individual samples. The assay was linear for serum dilutions ranging from 1:50 to 1:10,000 ($r=0.951$; $p<0.013$). For comparisons with a standard curve obtained by diluting human CSF from a responder, both pre-immune and immune serum samples were used at 1:50 dilutions and categorized by two independent and blind raters into the following 5 immunoreactivity corresponding to 1:10,000 (+), moderated, 1:5,000 (++); strong, 1:1,000, (+++); very strong, 1:500 (++++). To determine the increase in immunoreactivity during treatment, the pre-immune immunoreactivity scores were subtracted from immune scores to generate the following groups: no increase: $n=10$ including one death in placebo group equals $n=9$ observed cases in non-responder group. In the responder group ($n=20$), one patient dropped out because he was unwilling to participate in neuropsychological testing at month 12, leaving $n=19$ observed cases. To compare the degree of the immune response to the clinical outcome, this group was further subdivided into two groups bases upon the degree of increases in immunoreactivity scores as follows: Strong increases representing 4+ increases from pre-immune to immune status ($n=6$), and moderate increases representing the remaining group of 1+ to 3+ increases ($n=13$) from pre-immune to immune status.